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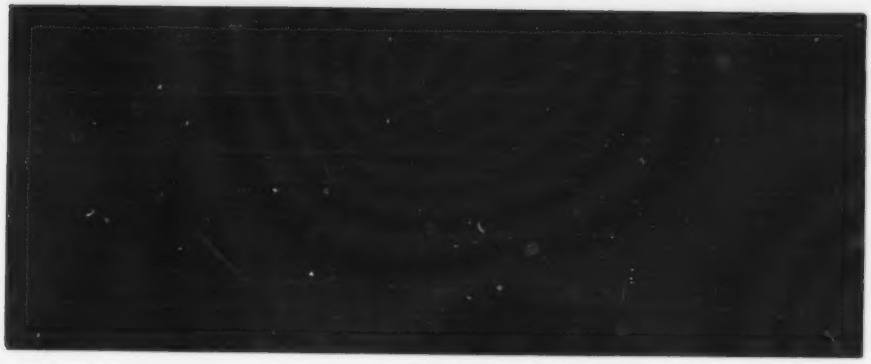

Final Report

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Metabolic Profiles and Hormone Responses of Growing Horses Reared in the Cold

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TECHNICAL REPORT: METABOLIC PROFILES AND HORMONE RESPONSES OF GROWING HORSES REARED IN THE COLD

ABSTRACT

Twelve, 7 month-old Standardbred colts were assigned to the following treatments: warm- or cold-housing. Warm-housed colts were kept in a barn set at a temperature of 10°C whereas cold-housed colts were kept in a barn whose temperature fluctuated with outdoor ambient temperatures. The mean temperature of the cold barn was -5.2°C over the study. Clincial biochemistry was measured at 4 (and 5)-week intervals beginning at week 1. Thyrotropin-releasing hormone (TRH) stimulation tests were conducted at weeks 10 (mid-February) and 21 (late April). Cold-housed horses had lower basal values for circulating glucose, creatinine, phosphorus, thyroxine (T_4) and triiodothryonine (T_3) but higher serum calcium concentrations and alkaline phosphatase activities than warm-housed colts. Basal T_4 and T_3 concentrations were positively related to age, but T_4 content was also related to ambient temperature of the barn. Basal T_4 was positively related to the energy cost of maintenance while basal T_3 relied on daily DE intakes. The TRH stimulation test indicated a reduction in T_4 and T_3 production in cold-housed horses in February compared to April. This may be due to thyroid gland suppression but not dysfunction of the pituitary-thyroid axis. Thyroid hormone secretion in young horses was seasonally dependent with possible modification by ambient temperature and energy status. Seasonal variation in thyroid hormone production may be an important regulator of bone growth in horses.

BACKGROUND AND OBJECTIVES

Young horses are assumed to be intolerant of cold weather, yet recent reports contradict this belief. When fed high-quality, pelleted diets free-choice, weanling horses consumed 30% more digestible energy (DE) than recommended for moderate growth. This high energy intake allowed a normal growth rate despite exposure to extreme cold (<-20°C) which caused maintenance energy requirements to increase by 50%. However, cold tolerance afforded by a high voluntary DE intake are negated by substandard feeding practices of young horses which occur not infrequently in Saskatchewan.

Metabolic effects of chronic cold exposure are poorly documented in horses. In ruminants, acute cold causes circulating thyroid hormones, blood glucose and free fatty acids to increase. Both triiodothyronine (T_3) and thyroxine (T_4) are associated with increased thermogenesis and energy expenditure during cold weather. The thyroid hormones not only regulate heat production but in horses may be important in potentiating growth hormone effects on the skeletal growth plate. That is, increases in thyroid hormones may facilitate bone growth. However, the pattern of thyroid hormone production is unknown for growing horses reared in cold weather.

The aims of the study were to compare the chronic biochemical changes and hormonal responses to stimulation with thyrotropin releasing hormone of colts reared in cold and warm barns during a typical, prairie winter.

EXPERIMENTAL METHODS

A split-plot experimental design was used to examine effects of housing temperature on metabolic responses of twelve 7-month old Standardbred colts for 22 weeks starting in late November. The treatments were: 1) a control

group (warm) which was housed in a barn set for a mean ambient temperature of 10°C, and 2) a treatment group (cold) which was kept in an uninsulated barn with no external heat supply.

Animals, Housing and Experimental Diets. A 6-week adaptation period was used from October to November when the colts were kept outdoors with access to an unheated shelter. During adaptation, a pelleted diet (Table 1) was fed at the rate of 1.25 kg/100 kg BW and brome-alfalfa hay was fed ad libitum. The colts were given ivermectin paste at 3-months intervals. Colts in the warm housed group were moved into the heated barn 1 week before the study started.

During the study, colts in both barns were fed a ration consisting of 75% pelleted diet and 25% coarsely chopped brome hay at the rate of 2.21 kg feed/100 kg BW daily (Table 1). This amount of feed met the nutrient requirements needed for moderate growth by 7-month old horses expected to mature at 500 kg BW. Feed intakes were adjusted weekly according to body weight. Any refusals were weighed daily and discarded.

Clinical Chemistry. Venous blood was drawn from the jugular vein into sterile, uncoated or heparinized evacuated tubes at week 1, and at 4-week intervals thereafter except for a final 5-wk interval. Blood for glucose was collected in tubes containing sodium fluoride and potassium oxalate. Blood was drawn after a 12 to 14 hour fast and was submitted for analyses immediately. All biochemical determinations were made on a DACOS analyzer using standard methods. Serum and plasma for T_4 and T_3 assays was stored at -20°C. These analyses were made by a fluorescence polarization immunoassay. The intraassay variation for the T_4 and T_3 analyses was 10 and 9%, respectively. Serum insulin was determined by radioimmunoassay methods.

TRH Stimulation Test. Synthetic thyrotropin-releasing hormone (TRH) was

dissolved in physiological saline to a final concentration of 0.5 mg/ml. The solution was prepared 1 hour prior to use. Blood was drawn before feeding and 2 hours post-feeding (pre-TRH injection), then TRH was injected intravenously at the rate of 2.5 ug/kg BW. Venous blood was taken at 15 minute intervals for the first hour, then at 20 minute intervals for the next 4 hours. Blood was centrifuged at 2000 g for 20 minutes at 4°C. T_4 and T_3 were determined in serum samples taken pre-feeding, 2 hour post-feeding (preTRH), at 1, 2, 3, and 4 hours post-TRH injection. Blood for glucose analyses were taken pre-feeding, 2 hour post-feeding (pre-TRH), at 30 minutes, 1, 2, 3, 4, and 5 hours after TRH injection. TRH stimulation tests were conducted at week 10 (mid-February) and 21 (late April) of the study.

Statistical Analyses. Statistical analyses included analysis of variance, repeated measures analysis of variance and correlation analysis. Univariate statistics were used to compare treatment effects within sampling period. In evaluation of correlations to temperature, only 6 observations per group were available which accounts for the lower level of significance obtained for this variable. For all other variables including age, the number of observations was 36 per group. Two colts in the cold-housed group in February and one colt in the warm-housed group in both periods showed atypical insulin responses. Insulin values of these colts were three to four-fold higher than those of the other horses. To compare treatment effects for insulin, an unbalanced analysis of variance was used excluding the data from these horses.

RESULTS AND DISCUSSION

Growth and Nutrient Intake. The mean temperatures were 10.9 ± 0.66 and -5.2 ± 1.72 °C for the warm and cold barn, respectively. Average daily gains of warm-housed colts at 0.67 kg/d were equal to values predicted in 1989 NRC tables. The lower daily gains (0.52 kg/d) of cold-housed colts were ascribed to a 29% increase in maintenance energy demands relative to those needed by colts kept at thermoneutral temperatures. The pertinent findings for nutrient digestion are summarized in Table 2. Housing temperature affected only fibre and phosphorus digestibility. Digestion of P also declined markedly with age. Gross energy and crude protein digestion declined from 8 to 12 months of age but Ca digestion did not change. In the present study, age effects were confounded by seasonal effects. However, because photoperiod and temperature effects were largely fixed, time variation was attributed to age.

Metabolic Profiles. The effect of housing treatment on the mean values of several biochemical variables are given in Table 3 and shown in Fig 1 and 2. Plasma glucose, serum creatinine and P concentrations were lower, but serum Ca concentrations and alkaline phosphatase activities were higher, in cold- compared to warm-housed colts. The concentrations of all variables changed over time. Except for week 5, circulating T_4 and T_3 concentrations were lower for cold- than for warm-housed colts. These changes are shown in Fig 3. Despite treatment differences, values for all biochemical variables except creatinine for cold-housed colts and alkaline phosphatase for both groups were within normal reference limits for horses of this age.

Plasma glucose concentration was inversely related to ambient temperature for cold-housed horses but was unrelated to temperature for warm-housed colts. In addition, plasma glucose content depended on basal T_4 concentration and

maintenance energy demand (Table 3). Serum creatinine concentration was lower for cold- than for warm-housed horses and was directly related to ambient temperature. Serum protein values declined with age.

The metabolic responses of cold-housed, growing horses were unlike observations made for cold-exposed cattle. In ruminants, cold stress elevates plasma glucose by stimulating gluconeogenesis. Yet, these cold-housed horses had lower basal glucose concentrations than their thermostable complements. The horse is intermediate between ruminants and non-ruminants in glucose production but basal values can vary with diet, age, breed and activity. In acute feeding studies, basal glucose concentrations of horses fed high, intermediate and low carbohydrate diets did not differ but diet produced profound effects on post-prandial glucose concentrations. By contrast, low basal glucose concentrations and the failure to produce a post-prandial glucose response in young horses fed 160% NRC energy intakes were ascribed to a high starch intake and its associated stimulation of insulin production. Yet, recently, a four-fold range in dietary starch intake by horses had no effect on blood glucose concentrations.

Glucose contributes more to energy metabolism of horses fed oats than hay. The dietary ingredients used in this study were 56% roughage and 44% concentrate. Some of the circulating blood glucose from this type of diet would be derived from the fatty acids produced in forage digestion. During fasting, energy metabolism in the horse depends on glucose and free fatty acid substrates. Therefore, the lower fasting glucose of cold-housed colts implies that more glucose was used for heat production. A low blood glucose would also be advantageous to the horse by promoting lipolysis and providing substrate for gluconeogenesis.

Serum Ca and P concentrations were within the normal reference limits for growing horses. Serum Ca concentration was higher but serum P content was lower for cold-housed colts compared to warm-housed animals. Serum P declined with age for warm-housed horses but not for cold-exposed colts. Alkaline phosphatase activities declined with age and increasing ambient temperature but were unrelated to serum Ca and P concentrations.

Serum P concentrations and total alkaline phosphatase activities in horses are high early in life and decline to adult values by 1 to 2 years of age. An unexpected finding in this study was the difference in serum Ca and P between cold and warm-housed colts. A 36% reduction in serum P content was reported for thyroidectomized fillies and was attributed to reduced feed intakes. However, the lower serum P content of our cold-housed horses likely resulted from their reduced P digestion. Recently, T_3 has been shown to potentiate intestinal P absorption. Thus, the reduction in blood P levels and P digestion may reflect the suppressed T_3 production in cold-exposed horses (see below).

High serum alkaline phosphatase activities are normal in young horses and indicate increased activity of the bone isoenzyme. Both groups had alkaline phosphatase activities above normal reference limits for horses of this age. Cold-housed colts had higher alkaline phosphatase activities than warm-housed colts implying more active and perhaps, abnormal bone or tissue metabolism. Since other tissue enzymes were normal (data not shown), the higher alkaline phosphatase activities likely originate from bone. There was no correlation between alkaline phosphatase activity and serum Ca and P concentration (Table 4) which might confirm bone pathology. Seasonal fluctuation and P deprivation have been linked to higher serum alkaline phosphatase activities in cattle.

Although basal T_3 and T_4 concentrations were lower for cold- than for warm-housed colts, both groups had normal thyroid hormone levels according to reference limits for growing horses. A direct association existed between maintenance energy expenditure, age, ambient temperature and basal T_4 content (Table 4). Circulating T_3 was not correlated to ambient temperature but rather was positively associated with DE intake, age and T_4 concentrations.

Serum T_4 and T_3 concentrations in horses are reported to vary with age, diet, season and ambient temperature. Generally, T_4 and T_3 concentrations are high in newborns and decline to adult values within 2 week (T_4) or 3 months post-natally (T_3). Age and breed can affect T_4 values in cattle and an age may affect T_4 values in horses. Age effects in cattle are felt to reflect a thyroxine-body weight dependence.

In studies with mature horses, peak T_4 values occurred in the fall and were inversely related to photoperiod but not temperature. These studies were subsequently confirmed in Canadian reports using mature, acclimatized horses whose basal T_4 and T_3 concentrations were found to increase during winter despite no changes in metabolic rates. Thyroxine concentrations increased in our horses as ambient temperatures increased which is opposite to the response observed for cattle. Our data agree with studies in horses, however, which reported increased plasma T_3 and T_4 during winter but not in relationship to ambient temperature. Thus studies with horses imply that thyroid hormone production is seasonally dependent and not temperature dependent as it is in cattle. The type of response seen in horses is often reported for feral ruminants such as deer who reduce thyroid hormone production possibly in relationship to low energy intakes and photoperiod changes.

Serum T_3 and T_4 content for horses were also apparently related to

indicators of energy metabolism. Elevation in T_4 as maintenance energy demands increase is compatible with the role of this hormone in increasing heat production. In cattle, subnormal energy intakes stimulate T_4 production during cold stress, but reduce T_4 concentrations in thermoneutral environments. This is opposite to our observations. Subnormal energy intakes also cause subnormal T_3 production in cold-exposed ruminants. The low thyroid hormone values in our cold-housed horses cannot be discounted as a response to subnormal energy intakes under the cold conditions to which these horses were exposed.

TRH Stimulation Test. The T_3, T_4, insulin and glucose responses of the warm and cold-housed colts to TRH injection are shown in Fig. 4 and 5. Univariate analyses of data indicate that treatment significantly affected T_4 and glucose production. Period (February versus April) effects occurred for glucose, T_3, and T_4 but not for insulin. Secretion of all hormones and glucose changed after TRH stimulation.

Warm-housed colts had higher T_4 values at 1 and 4 hours after TRH injection than cold-housed colts. In April, T_4 concentrations following TRH stimulation were higher at 2 and 3 hour post-injection than at these times in February. There were no treatment effects for T_3 although fasting values were higher in April than in February. The T_3 values at 1 and 2 hours after TRH injection were higher in April than in February. Fasting plasma glucose concentrations were higher in April than February and were higher for warm than cold-housed colts. A period effect (April vs February) occurred 2 hour after TRH injection. Fasting or basal insulin concentrations were lower in February than in April but did not differ between treatments. Treatment differences in insulin response were observed at 100, 140 and 180 minutes after TRH injection.

The TRH stimulation test has been used in horses to diagnose thyroid dysfunction or dysfunction of the pituitary-thyroid axis. The normal response of horses to TRH injection is a three-fold elevation in T_3 at 2 hours, followed by a two-fold elevation in T_4 concentrations at 4 hours after injection. Route of injection and amount of TRH administered alter the pattern and magnitude of the response. Mares given 0.5 mg TRH showed a two-fold increase in T_4 concentration 2 hours after injection. In the present study, the dose used was 2.5 ug TRH/kg BW which was administered two hour after eating. The maximal T_3 peak was obtained 1 to 2 hour following TRH injection at concentrations two- to three-fold above post-prandial values. By contrast, T_4 continued to increase up to 4 hours after injection. Maximal T_4 concentrations were only 25 to 40% higher than post-prandial values.

The response of glucose and insulin to TRH stimulation was confounded by the marked post-prandial elevation in glucose and insulin concentration. Peak glucose values occurred 2 hour post-feeding and preceded TRH injection. Insulin secretion peaked 15 to 30 minutes later than glucose. The only differences in glucose concentrations between groups occurred at fasting and 2 hours after feeding. The lower glucose concentrations of the cold-housed colts may be the result of enhanced use of glucose during prolonged cold. Treatment or housing effects did not alter insulin secretion although a treatment X period interaction was found. Insulin secretion of each group differed in February and April. Warm-housed colts maintained a high insulin concentration for 2 hours longer than cold-housed colts in February. Therefore, insulin production was increased or insulin use was reduced in warm-housed horses during this period. The higher insulin levels did not appear to hasten the rate of T_4 disappearance in our horses as suggested by others.

The TRH stimulation tests indicated that thyroid function was suppressed by cold temperatures or seasonal (photoperiod) effects. The lowered T_3 secretion by cold-housed than warm-housed horses in February but also, the lower February than April response, supports both a seasonal and temperature effect. By contrast, T_4 secretion after TRH stimulation showed a trend, although small, to temperature or housing differences.

The subnormal production of T_3 and T_4 in the cold-housed group in February, possibly evinced by cold weather, was followed by an elevation in thyroid hormones during warming in April. An important thyromimetic activity is stimulation of long bone growth. Therefore, reduction in serum T_3 and T_4 during prolonged cold stress may retard bone growth in young horses. The importance of the thyroid hormones in equine skeletal growth has been recognized following association of hypothyroidism to limb abnormalities in foals. If thyroid hormone production is suppressed during winter and is further suppressed by feeding high carbohydrate diets as suggested by some authors, then abnormal replication and maturation of the growth plate would be expected in yearling horses. A seasonal change in hormone production may explain the growth spurts and leg abnormalities seen at specific ages in horses. Contracted tendons and physitis commonly occur just after weaning and at about 1 year of age. In the spring in addition to elevated thyroid hormone production there is amelioration of cold weather. Thus, less dietary energy is needed for maintenance heat production and therefore, can be used in tissue anabolism. A combination of higher (although normal) thyroid hormone production coupled with an increase in net energy for growth may contribute to abnormal development of the cartilaginous growth plate and hence, abnormal bone development in yearling horses.

CONCLUSIONS AND IMPACT

1. Weanling horses fed at 1989 NRC nutrient recommendations but kept in unheated barns during a typical Saskatchewan winter had metabolic profiles that indicated subnormal energy balance. Cold stress metabolically aggravated energy balance of overwintered weanlings fed a "normal" energy intake. This substantiates our previous conclusions that 1989 NRC recommendations for energy are inadequate for Saskatchewan winter conditions.

2. Cold-housed weanlings had higher blood calcium and lower blood phosphorus concentrations than those kept in warm barns. Thus, bone quality may differ in young horses kept in the cold but does not confirm that bone quality is worse. This data further supports increasing dietary phosphorus concentration from 0.30% to 0.35% or 0.40% for cold-housed, weanling horses.

3. Cold-housed weanlings secreted less thyroid hormone in response to cold temperatures. Thus these horses fed at "normal" energy intakes according to 1989 NRC guidelines responded to cold stress like wild ruminants such as deer, but not like cattle. Low thyroid hormone production may indicate subnormal energy intake, cold intolerance or an atypical metabolic adaptation to cold. Increasing dietary energy or changing the diet to a higher proportion of forage may benefit horses thermodynamically by increasing heat increment and thus, their cold tolerance. This hypothesis remains to be confirmed.

4. Thyroid function of weanlings kept in a cold barn was sluggish throughout the winter. This effect was confirmed by TRH stimulation. Low production of thyroid hormones has two implications: 1) a decreased cold tolerance in young

horses kept outdoors in winter and 2) a possible adverse effect on bone development since thyroid hormones potentiate skeletal growth by acting permissively with growth hormone. Diet and housing may prove to be important determinants of leg disorders in horses.

5. Weanling horses overwintered outdoors in Saskatchewan may develop bone subnormally for several reasons: inadequate energy intake, reduced mineral absorption and reduced thyroid hormone production. Yet, in contrast to expectation, cold-housed horses had a lower incidence of leg disorders over winter. Whether these skeletal effects were permanent or temporary is unresolved. Seasonal variations in hormone production may explain why leg disorders such as epiphysitis and contracted tendons occur at specific ages. These possibilities must be further examined since they potentially represent an exciting solution to a chronic problem of young horses reared in the Prairies.

Table 1. Ingredient and nutrient composition (%) of pelleted diet and brome hay used in the study

	Pelleted diet	Brome Hay
Ingredient composition, %		
Alfalfa-brome hay	41.0	
Barley	21.0	
Oats	30.0	
Soybean meal	5.0	
Tallow	1.0	
Sodium monophosphate	0.5	
Iodized salt	0.5	
Mineral-vitamin premix[a]	1.0	
Nutrient Composition		
Crude protein, %	15.54	10.72
ADF, %	22.01	39.36
NDF, %	41.34	59.90
Calcium, %	.72	.65
Phosphorus, %	.48	.24
GE, Mcal/kg	4.55	4.37

[a] The premix contained (per kg):0.12% copper, 0.26% zinc, 0.23% manganese, 250,000 IU vitamin A, 27,000 IU vitamin D and 1500 IU vitamin E in ground barley.

Table 2. Nutrient digestibilities[f] (%) of the ration (75% pelleted diet and 25% brome hay as fed)

Item	Age, months			SE
	8	10	12	
Energy	62.9[a]	59.7[b]	57.2[c]	0.50
Crude protein	77.8[a]	76.2[a]	73.5b	0.40
Acid detergent fibre[§], warm	25.3[a]	20.0[b]	12.8[c]	0.79
cold	23.5[b]	29.6[a]	18.8[c]	
Calcium*	68.2	73.2	68.8	0.80
Phosphorus*[§], warm	52.2[a]	42.8[b]	34.8[c]	1.50
cold	46.9[a]	39.2[b]	27.1[c]	

f Values are apparent digestibilities except for calcium and phosphorus.

* Calcium and phosphorus digestibilities are corrected corrected for endogenous fecal calcium and phosphorus losses of 20 and 10 mg/kg body, respectively.

[a-c] Values with different superscripts within row are different at $P < 0.05$.

[§] Values for warm-housed and cold-housed colts differ at $P < 0.05$.

Table 3. Mean metabolic profiles of cold and warm-housed horses over 22-wk.

Biochemical variable[§]	Warm	Cold	Normal Range[*]
Sodium, meq/L	138.7	139.7	135 - 148
Potassium, meq/L	4.0	4.0	3.5 - 5.0
Chloride, meq/L	97.9	98.1	98 - 110
Carbon dioxide, meq/L	29.0	28.6	20 - 32
Calcium, mmole/L[ƒ]	2.94	3.02	2.8 - 3.4
Phosphorus, mmole/L[ƒ]	2.36	2.26	0.7 - 1.7
Creatinine, mmole/L[ƒ]	112.4	100.7	110 - 170
Glucose, mmole/L[ƒ]	5.32	5.07	3.6 - 5.6
Protein, mmole/L	61.0	60.6	60 - 77
Albumin, mmole/L[ƒ]	31.6	32.5	25 - 36
Alkaline phosphatase, U/L[ƒ]	361.8	415.7	95 - 233
Creatine phosphokinase	254.8	260.6	0 - 500
Gamma glutamyl transpeptidase, U/L	13.8	13.6	0 - 25

§ All variables had a significant ($P<0.01$) time effect.

* Laboratory values are based on adult horses.

ƒ Values for warm-housed colts differ ($P< 0.05$) to cold-housed colts.

Table 4. Relationships between certain metabolic variables based on Pearson's correlation coefficients

Biochemical Variable	Temperature[f]	Period (Age)	DE Intake	Maintenance Intake	T3	T4
Glucose	-.49* (C)	-.16	-.18	-.13	.04	.30
T_3	.18	.51***	.40***	.01	-	.44***
T_4	.54*	.61***	.26**	.30**	.44***	-
Creatinine	.79***	.33***	.45***	-.05	.33***	.36***
Protein	-.21	-.55***	-	-	-.03	-.20
Calcium	-.33	.21*	.23*	.16	.08	.09
Phosphorus	-.12	-.24*	-.07	.17	-.03	.04
Alkaline Phosphatase	-.88***	-.32**	.13	.15	-.17	-.02

[f] Values for temperatures are based on 6 observations per group; all other observations are based on 72 values for data of both groups.

C = cold-housed group.

* P<0.1
** P<0.05
*** P<0.01

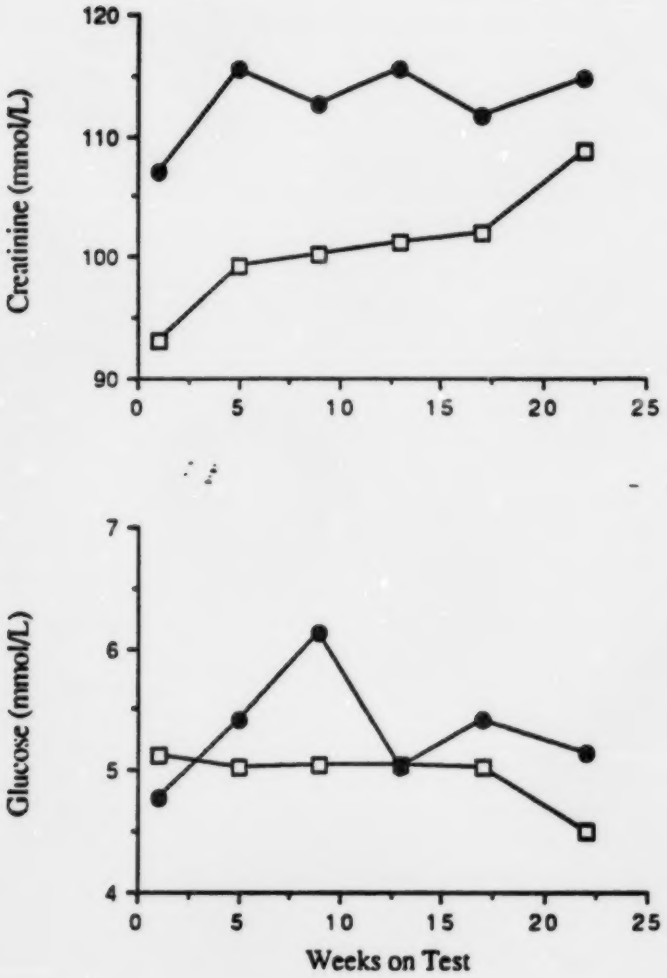

Figure 1. Changes in plasma glucose and creatinine content in warm (●) and cold (□) -housed colts over winter

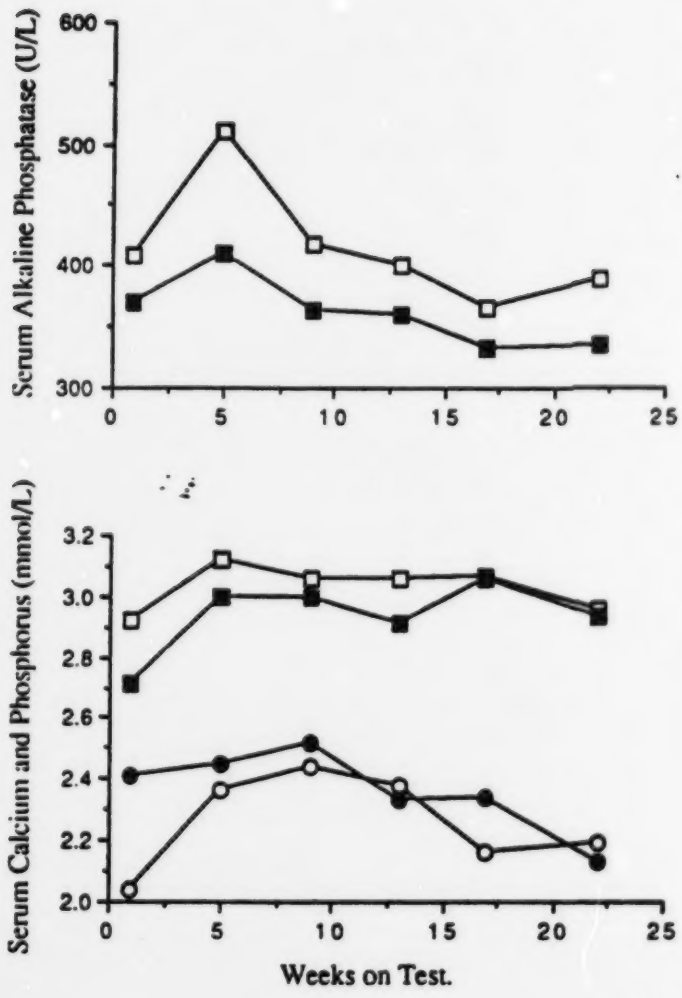

Figure 2. Changes in serum alkaline phosphatase activity of warm (■) and cold (□)-housed colts over winter and the associated changes in serum calcium concentration of warm (■) and cold (□) housed colts and serum phosphorus concentration of warm (●) and cold (○)-housed colts.

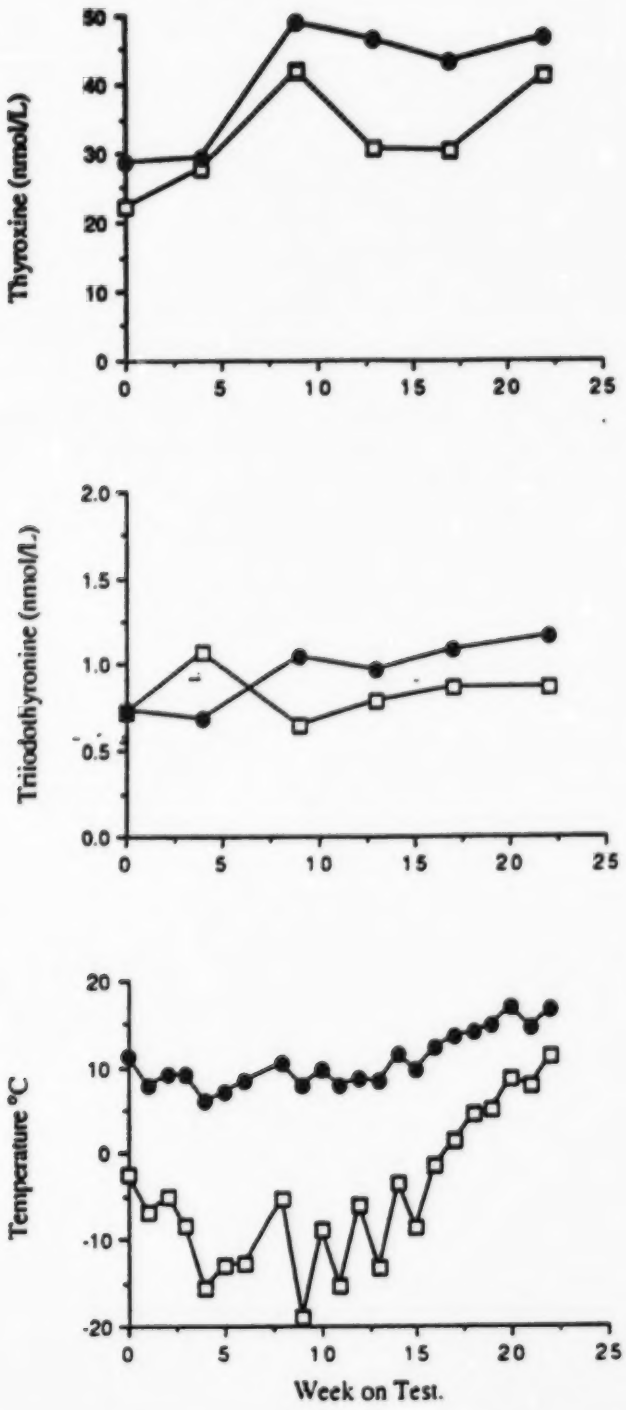

Figure 3. Changes in fasting thyroxine and triiodothyronine concentrations of warm (●) and cold (□)-housed colts over winter. Barn temperatures are also shown.

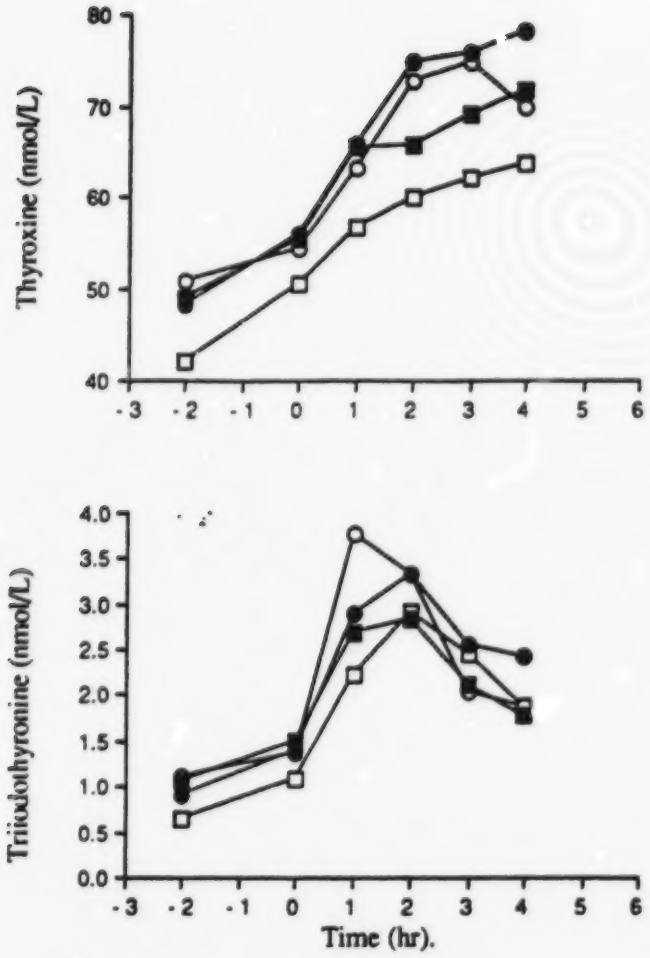

Figure 4. The effect of TRH stimulation on thyroxine and triiodothyronine responses of cold-housed colts in February (□) and April (○) and of warm-housed colts in February (■) and April (●).

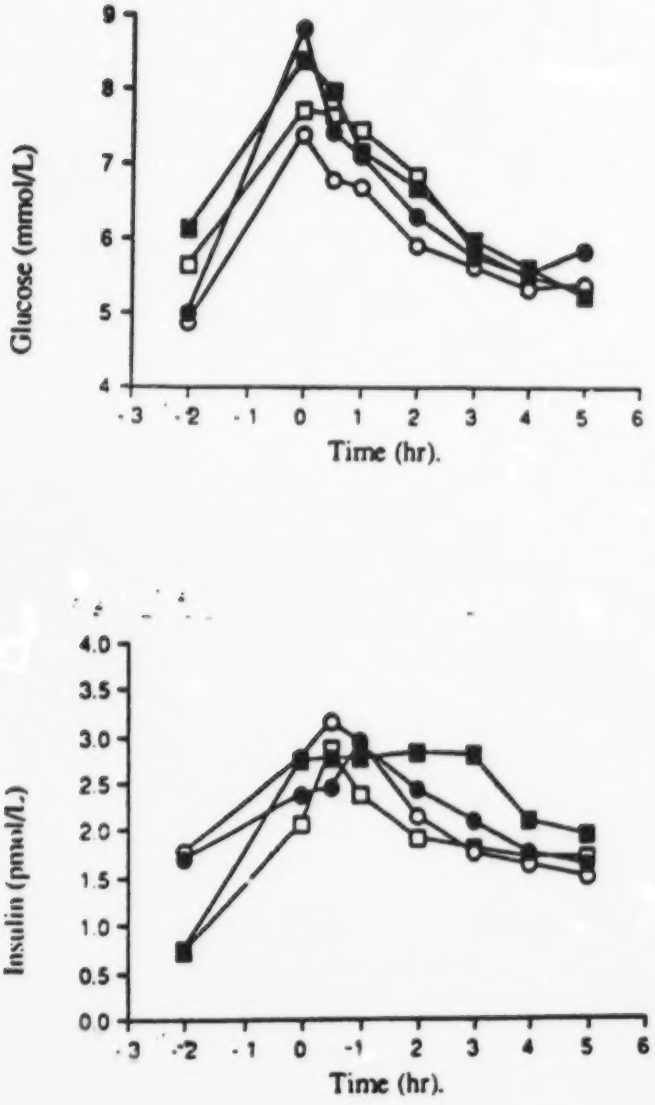

Figure 5. The effect of season and housing temperature on insulin and glucose responses to TRH stimulation. In the figure, warm-housed colts in February are shown as (■); warm-housed colts in April are (●); cold-housed colts in February are shown as (□) and cold-housed colts in April are shown as (○).

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